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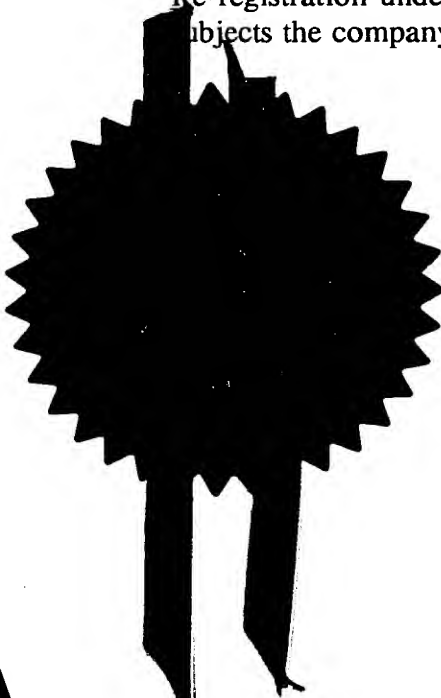
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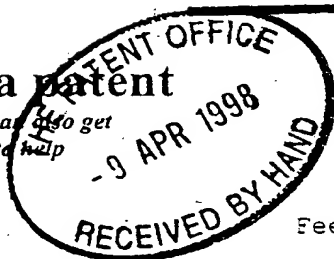
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1. Your reference	JMD/39437		
2. Patent application number (The Patent Office will fill in this part)	09 APR 1998	9807777.9	
3. Full name, address and postcode of the or of each applicant (underline all surnames)	Mars U.K. Limited 30 Dundee Road Slough Berkshire SL1 4LW	BTIG Wira House West Park Ring Road Leeds LS16 6QL	
Patents ADP number (if you know it)	5632260001	7415078001	
If the applicant is a corporate body, give the country/state of incorporation	UNITED KINGDOM	UNITED KINGDOM	
4. Title of the invention	ADHESIVES		
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Adhesives

The present invention relates to water resistant adhesives.

5 Adhesives are widely used both domestically and industrially. They are typically applied to dry first and second surfaces to bond the surfaces together, for example as bonding agents to bond together particulate matter, or to adhere other solid materials such as woods and metals. It is often desired to bond surfaces together when one or more of the surfaces is wet. Many adhesives are, however, less effective or ineffective if the surfaces that are to be bonded together are wet or if water is applied to the bond after it has been formed. Consequently, much effort has been spent trying to identify and develop adhesives which are effective in wet environments.

Marine mussels are able to attach themselves to a variety of surfaces under water forming strong and durable bonds with those surfaces. The precise mechanism by which mussels achieve this adhesion is not known. J.H.Waite has described the proposed involvement of catechol oxidase and a phenolic protein (Mussel Beards: a coming of age. Chemistry & Industry, 1991, 607-611). The mussel polyphenolic protein is a highly repetitive protein which comprises 80 tandem repeats of a decapeptide of the sequence: Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys. The mussel protein is particularly rich in the amino acid 3,4-dihydroxyphenyl-L-alanine (L-dopa). Catechol oxidase occurs extensively throughout nature and is known to catalyse the ortho hydroxylation of phenols and oxidation of the resulting catechols to o-quinones. Waite proposed, therefore, that catechol oxidase catalyses oxidation of the L-dopa residues in the mussel polyphenolic protein to L-quinone. It was suggested that adhesion results from a combination of coupling of peptidyl-dopa to the bound surface and crosslinking of the polyphenolic protein by

reaction of peptidyl-dopa-quinone with nucleophiles such as the ϵ -amino group of lysine residues in the protein.

A catechol oxidase from mushrooms known as mushroom tyrosinase is available commercially and has been shown to
5 hydroxylate tyrosine residues in synthetic decapeptides identical in sequence to the repeat sequences in the polyphenolic protein (Marumo K. and Waite J.H. (1986) Optimization of hydroxylation of tyrosine and tyrosine-containing peptides by mushroom tyrosinase. *Biochemica et*
10 *Biophysica Acta* 872, 98-103).

Large scale production of the mussel polyphenolic protein has been attempted with a view to production of a commercial adhesive which can be crosslinked by mushroom tyrosinase. Methods for isolation of the polyphenolic
15 protein from mussels are described in US 4 496 397, US 4 585 585, and US 4 687 740. None of these methods, however, have proved to be commercially viable because the polyphenolic protein can only be isolated in small quantities.

WO 97/19141 describes a method of manufacture of an adhesive comprising crosslinking first and second molecules such as gelatin and chitosan, each having one or more aromatic groups, via at least one quinone group. Gelatin and chitosan are readily available and adhesives formed by
25 this method show some resistance to water. However, the bonds formed are not as strong and durable as those formed by the mussel polyphenolic protein. A further disadvantage is that gelatin is not soluble in water at room temperature and has to be heated before use. Chitosan is only soluble
30 at low pH.

It is desired therefore to produce bioadhesives which are effective in wet environments, which can be produced cheaply in bulk quantities, and which have improved properties over known readily produced bioadhesives.

35 Extensin proteins are hydroxyproline-rich glycoproteins present in the cell walls of dicotyledonous plants. The exact function of extensins is not known, but they have been proposed to play a role in the structure of plant cell walls. Extensins also accumulate in plant cell walls upon

wounding and pathogenic attack and are therefore also thought to be involved in defence. It is known that extensins are inherently sticky and their adhesion to glass, polypropylene, and polycarbonate has been described in a paper by Miller, J.G. and Fry, S.C., (1993) (Spinach extensin exhibits characteristics of an adhesive polymer. *Acta Bot. Neerl.* **42**(2), 221-231). However, the mechanism of adhesion here appears to be a mixture of purely intramolecular forces such as hydrogen bonding and ionic interactions. Oxidative processes are not involved as the adhesion is not inhibited using reducing agents such as ascorbate, mercaptoethanol and dithiothreitol. Although native non-crosslinked proteins can adhere to inert substances, the set adhesive will be sensitive to moisture. Ultimately strength loss and bond breakage will become apparent. A successful commercial adhesive system must be crosslinked both to improve the strength of the adhesive and to ensure adhesion in wet environments and areas of high moisture.

Extensins have been proposed to form crosslinks by Fry, C.F., (1982) (Isodityrosine, a new crosslinking amino acid from plant cell wall glycoprotein. *Biochem.J.* **204**, 449-455). Fry suggested that hydrogen peroxide and a peroxidase enzyme such as horseradish peroxidase could be used to form isodityrosine bonds via an ether linkage between two tyrosine residues. Formation of dityrosine crosslinks via a biphenyl linkage between two tyrosines was not thought to occur. The formation of an isodityrosine bond and comparison of that linkage with the dityrosine linkage is represented in figure 1 of the accompanying drawings.

The proposed existence of such crosslinking suggests that extensins may form adhesives in the presence of hydrogen peroxide and a peroxidase enzyme. However, it is most unlikely that formation of an adhesive based on addition of a cofactor such as hydrogen peroxide will be practical. Hydrogen peroxide is a relatively low viscosity liquid and would be very difficult to mix with other components of the adhesive which also tend to be relatively viscous. Hydrogen peroxide is also fairly reactive and it

is likely that bond formation would occur too quickly after it is added to the other components of the adhesive. A further disadvantage of hydrogen peroxide is that it has relatively short shelf-life. Any contamination from, for example, dirt would introduce bacteria, many of which contain catalase enzymes which breakdown hydrogen peroxide.

Peroxidase has also been proposed to be involved in the hydroxylation of L-tyrosine residues to L-Dopa (Klibanov A.M., Berman Z., and Alberti B.N. (1981) Preparative hydroxylation of aromatic compounds catalysed by peroxidase. *J.Am.Chem.Soc.* 103, 6263-6264), suggesting an alternative role for peroxidase in crosslink formation similar to crosslink formation in the mussel polyphenolic protein adhesive. However, this reaction requires oxygen and dihydroxyfumaric acid and must be carried out at 0°C, otherwise non specific oxidation of other amino acids occurs. Use of peroxidase for crosslinking of extensin proteins has therefore not been thought to provide a viable way of producing a commercial adhesive.

It has surprisingly been found that extensin proteins have remarkable adhesive properties.

According to a first aspect of the invention there is provided a composition for use as an adhesive comprising:

an extensin protein; and either

a non-enzymatic bifunctional crosslinking agent; or a phenol oxidase and a phenol hydroxylase.

According to a second aspect of the invention there is provided a composition for use as an adhesive comprising:

an extensin protein;

a phenol oxidase and a phenol hydroxylase; and a non-enzymatic bifunctional crosslinking agent.

According to the invention there is also provided a method for forming an adhesive according to the first aspect of the invention which comprises admixing an extensin protein with either:

an amount of a non-enzymatic bifunctional crosslinking agent; or

an amount of a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

Also according to the invention there is provided a method for forming an adhesive according to the second aspect of the invention which comprises admixing an extensin protein with an amount of a non-enzymatic bifunctional crosslinking agent, a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

Each component of compositions according to the invention may be soluble in water.

When using a phenol oxidase and a phenol hydroxylase for crosslinking, it is possible to include as a cofactor a phenolic moiety which comprises at least one of a monohydroxy phenol group or a dihydroxy phenol group. Examples of phenolic moieties which comprise a dihydroxy phenol group include catechol and catechin. The cofactor should be soluble in water. The cofactor may be present at about 1% weight by volume of the composition.

The term "extensin protein" used herein is defined for the purposes of this application as covering:

(i) natural extensin proteins, such as plant extensins (for example carrot, spinach, etc.);

(ii) non-natural synthetic extensins, such as extensins produced chemically or by expression of recombinant DNA in a suitable host;

(iii) extensin derivatives (whether chemical or synthetic) which have amino acid sequences which differ from the extensin sequences by virtue of amino acid substitution, deletion, or addition, protease truncation or post translational modification; but which retain extensin activity.

Natural extensin protein as referred to in (i) above is a plant protein rich in hydroxyproline, tyrosine and lysine residues. The content of hydroxyproline in carrot extensin is at least 50%, the content of tyrosine is at least 10.1%, and the content of lysine is at least 6.9%.

DNA encoding carrot extensin has been cloned (Chen J. and Varner J.E. (1985) An extracellular matrix protein in plants: characterisation of a genomic clone for carrot extensin. EMBO J. 4, 2145-2151; Chen J. and Varner JE (1985)

Isolation and characterisation of cDNA clones for carrot
extensin and a proline-rich 33KDa protein.
Proc.Natl.Acad.Sci.USA 82, 4399-4403).

Derivatives of the extensin as referred to in (iii)
5 above may be obtained by expression of a modified DNA
including a modified extensin gene. A derivative of the
extensin may be substantially free of carbohydrate.

The extensin protein may be present in the composition
in an amount upto about 50% weight by volume of the
10 composition, for example, the extensin protein may be
present in the composition in an amount from about 20% to
about 30% weight by volume of the composition.

A procedure for isolation of an extensin according to
the invention from carrots is described below:

15 Carrot extensin proteins were isolated from cores (7mm)
of phloem parenchyma sliced with a scalpel into 1mm thick
slices. Carrot preparations (90g) (finely chopped or gently
homogenised) were washed twice in distilled water and
incubated in potassium phosphate buffer (5mM, pH 6.0)
20 containing chloramphenicol (50mg/ml) for three days at 28°C
with shaking. The extraction buffer was exchanged each day.
On completion, the carrots were washed in distilled water
and immersed in a solution (600ml) containing polyvinyl
polypyrrolidone (PVPP) (9g) and dithiothreitol (DTT) (5mM,
25 final concentration). The tissue was homogenised for two
minutes in a blender, pelleted by centrifugation and washed
with eight litres of distilled water. The cell wall pellet
was further extracted (3 times) with 200ml of a solution
containing calcium chloride (0.2M final concentration), DTT
30 (5mM final concentration) and PVPP (9g). On centrifugation
the supernatants were pooled, filtered to remove any solid
material and reduced to a volume of 50ml using
ultrafiltration (10,000 molecular weight cut-off). The
concentrated extract was dialysed overnight in distilled
35 water at 4°C and further reduced in volume to 10ml by
ultrafiltration. Each 10ml volume was adjusted with 1M
tris/HCl (pH 8) to give a final tris concentration of 10mM.
The material was then applied to a cation exchange column
(12 x 1.5 cm) (CM-Sepharose CL-6B) previously equilibrated

with tris/HCl (10mM, pH 8) and the protein fractions eluted using one column volume of tris/HCl (pH 8) followed by a linear gradient (60ml) of 10-300mM tris/HCl (pH 8). The elution profile was monitored at 280nm and the fractions
5 (3ml) corresponding to each peak were pooled and dialysed overnight with sodium acetate (100mM, pH 6) at 4°C. The dialysed samples were freeze-dried and weighed before further analysis. In all purifications peak 1 from the cation exchange column was ignored as it was primarily made
10 up of PVPP.

It is noted herein that peroxidases are not phenol oxidases. Peroxidases act by hydrolysing hydrogen peroxide. It is possible that oxygen liberated by this reaction can oxidise phenolics, but this oxidation does not occur by an
15 enzymatic process. Phenol oxidation is not possible in the presence of a peroxidase without the addition of hydrogen peroxide. It is also noted herein that peroxidases are not phenol hydroxylases.

The phenol oxidase and the phenol hydroxylase may be a
20 tyrosinase. The composition can contain at least 0.005% weight by volume of tyrosinase. Tyrosinase has the advantage that it is readily available from a variety of sources. The tyrosinase may be a mushroom tyrosinase. The mushroom tyrosinase may be *Agaricus bisporus* tyrosinase.

25 The non-enzymatic bifunctional crosslinking agent may be present in the composition from about 0.1% to about 5% weight by volume of the composition. The non-enzymatic bifunctional crosslinking agent may be any non-enzymatic bifunctional crosslinking agent capable of inducing
30 crosslinking of a composition for use as an adhesive according to the invention. The non-enzymatic bifunctional crosslinking agent may be at least one of glutaraldehyde, a di-isocyanate, or a quinone. The di-isocyanate may be Trixene, for example Trixene BI 7986 (Baxenden). Trixene
35 has the advantage that it is blocked and unreactive until the temperature reaches 130°C. Compositions according to the invention that comprise Trixene may therefore form a thermo-setting adhesive. The quinone may be a benzoquinone or a derivative thereof. The amount of the benzoquinone in the
40 composition can be about 1% weight by volume of the

composition. The benzoquinone may be 1,2-benzoquinone, 1,3-benzoquinone, or 1,4-benzoquinone.

It has been found that the rate of oxidation of group may be conveniently controlled by varying the amount of phenol oxidase and phenol hydroxylase that is added, allowing the rate of linkage to be readily controlled. The rate of linkage may also be controlled by varying the amount of the non-enzymatic bifunctional crosslinking agent.

When compositions according to the invention that contain the non-enzymatic bifunctional crosslinking agent form crosslinks, the non-enzymatic bifunctional crosslinking agent reacts with the ϵ -amino group of lysine residues in the extensin protein. Each molecule of non-enzymatic bifunctional crosslinking agent can react with upto two lysine residues. When the two lysine residues are in different protein molecules, crosslinks are formed between different protein molecules. When the two lysine residues are in the same protein molecule, crosslinks are formed within the same protein molecule.

When compositions according to the invention that comprise the phenol oxidase and the phenol hydroxylase form crosslinks, the phenol hydroxylase catalyses the hydroxylation of the phenol group of tyrosine residues in the protein to form a dihydroxy phenol group and the phenol oxidase catalyses the oxidation of the dihydroxy phenol group to form a quinone group. The quinone group of the modified tyrosine residue may then react with the ϵ -amino group of two lysine residues in the protein. When one or both of the lysine residues are in the same protein molecule as the modified tyrosine residue containing the quinone group, intramolecular crosslinking occurs. When one or both of the lysine residues are in different protein molecules to the modified tyrosine residue containing the quinone group, intermolecular crosslinking occurs.

Tyrosinase has the advantage that it is a phenol hydroxylase and a phenol oxidase. It will be appreciated therefore that compositions according to the invention which comprise tyrosinase have the advantage that a separate phenol oxidase and a phenol hydroxylase do not have to be

added to the composition because the phenol oxidase and the phenol hydroxylase activity are on a single molecule.

The hydroxylation and oxidation of a tyrosine residue by tyrosinase is shown in figure 2 of the accompanying drawings. Figure 2 also shows reaction of the oxidised tyrosine residue with the ϵ -amino group of a lysine residue in the same protein molecule.

When compositions according to the invention that comprise the phenol oxidase and the phenol hydroxylase further include a cofactor which comprises a phenolic moiety with a monohydroxy phenol group, the phenol hydroxylase catalyses the hydroxylation of the monohydroxy phenol group in the cofactor to form a dihydroxy phenol group and the phenol oxidase catalyses the oxidation of the dihydroxy phenol group to form a quinone group. The quinone group of the modified cofactor may then react with the ϵ -amino group of lysine residues in the protein to form crosslinks within the same protein molecule or between different protein molecules.

When compositions according to the invention that comprise the phenol oxidase and the phenol hydroxylase further include a cofactor which comprises a phenolic moiety with a dihydroxy phenol group, the phenol oxidase catalyses the oxidation of the dihydroxy phenol group of the cofactor to form a quinone group. The quinone group of the modified cofactor may then react with the ϵ -amino group of lysine residues in the protein to form crosslinks within the same protein molecule or between different protein molecules. Thus, a cofactor comprising a monohydroxy phenol group or a dihydroxy phenol group may be used to increase the number of crosslinks formed compared to compositions according to the invention which comprise the phenol oxidase and the phenol hydroxylase that do not include the cofactor. The number of crosslinks which can potentially be formed may also be increased by increasing the number of tyrosine and/or lysine residues in the extensin protein by recombinant means. However, care should be taken not to increase the number of crosslinks formed too much as over-crosslinked adhesives can be brittle.

It will be appreciated that compositions according to the invention that comprise a cofactor which includes a dihydroxy phenol group may form crosslinks in the absence of the phenol hydroxylase. Consequently, there is also

5 provided according to the invention a composition for use as an adhesive which comprises:

an extensin protein;

a cofactor comprising a dihydroxy phenol group;

a phenol oxidase; and optionally

10 a non-enzymatic bifunctional crosslinking agent.

There is also provided a method for forming an adhesive which comprises admixing an extensin protein with an amount of a cofactor comprising a dihydroxy phenol group, a phenol oxidase, and optionally a non-enzymatic bifunctional crosslinking agent effective for inducing crosslinking of the protein.

According to the invention there is also provided a kit for manufacture of an adhesive, the kit comprising separate components, wherein admixture of the separate components forms a composition according to the invention.

According to the invention there is also provided a kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising: either
25 a non-enzymatic bifunctional crosslinking agent; or
a phenol oxidase and a phenol hydroxylase and optionally a cofactor;

wherein admixture of the first and second components forms a composition according to the invention.

Also according to the invention there is provided a kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising a non-enzymatic bifunctional crosslinking agent and a phenol oxidase and a phenol hydroxylase and optionally a cofactor, wherein admixture of the first and second components forms a composition according to the invention.

Adhesives formed using compositions according to the invention have excellent strength, durability and water resistant properties. They are readily produced in bulk

quantities and at low cost. The strength of adhesives according to the invention has been found to be at least as good as the strength of conventional water resistant adhesives.

5 Adhesives according to the invention have been found to have several uses. Adhesives according to the invention may adhere to water-absorbent substrates and can be used to adhere such substrates together. Adhesives of the present invention may adhere to a substrate having a hydroxy
10 aromatic, dihydroxy aromatic, hydroxy phenone or amino group and can be used to adhere such substrates together. For example the substrate may be wood, leather, cotton, paper, carpet, or a textile.

15 Adhesives according to the invention have also been found to adhere to non water-absorbent substrates and can be used to adhere non water-absorbent substrates to each other or to water absorbent substrates. For example, the non water-absorbent substrate may be a metal or a plastic.

20 Adhesives according to the invention may be used to bind together particulates. Examples of particulates include sand and glass fibre.

25 Adhesives according to the invention may be used as an undercoat to a protective coating such as a paint or to a coating such as a non water-resistant adhesive. Undercoats comprising adhesives according to the invention have been found to have the advantage that the coating that is
30 subsequently applied to the undercoat is significantly more resistant to the effects of water than coatings applied in the absence of the undercoat or to coatings applied to a conventional undercoat.

35 Adhesives according to the invention may be used as a suture to close wounds. Sutures formed according to the invention have the advantage that they are resistant to water and may have reduced antigenicity compared to conventional sutures.

Adhesives according to the invention may be used as a gelling agent in food products.

According to the invention there is also provided a pharmaceutical composition comprising a pharmaceutically

active ingredient and a crosslinked adhesive composition according to the invention.

Claims

1. A composition for use as an adhesive comprising:
an extensin protein; and either
a non-enzymatic bifunctional crosslinking agent; or
a phenol oxidase and a phenol hydroxylase.
2. A composition for use as an adhesive comprising:
an extensin protein;
a non-enzymatic bifunctional crosslinking agent; and
a phenol oxidase and a phenol hydroxylase.
3. A composition according to claim 1 or 2 which further
comprises a cofactor when the composition comprises a phenol
oxidase and a phenol hydroxylase.
4. A method for forming an adhesive which comprises
admixing an extensin protein with either:
an amount of a non-enzymatic bifunctional crosslinking
agent; or
an amount of a phenol oxidase and a phenol hydroxylase
effective for inducing crosslinking of the protein.
5. A method for forming an adhesive which comprises
admixing an extensin protein with an amount of a non-
enzymatic bifunctional crosslinking agent, a phenol oxidase
and a phenol hydroxylase effective for inducing crosslinking
of the protein.
6. A method for forming an adhesive which comprises
admixing an extensin protein either with an amount of a
cofactor, a phenol oxidase and a phenol hydroxylase
effective for inducing crosslinking of the protein or with
an amount of a cofactor, a non-enzymatic bifunctional
crosslinking agent, a phenol oxidase and a phenol
hydroxylase effective for inducing crosslinking of the
protein.

7. A composition or method according to claim 3 or 6 in which the cofactor comprises a phenolic moiety which comprises at least one of a monohydroxy phenol group or dihydroxy phenol group.
- 5 8. A composition or method according to claim 3, 6, or 7 in which the cofactor is soluble in water.
9. A composition or method according to claim 7 or 8 in which the cofactor comprises catechin.
- 10 10. A composition or method according to any of claims 7 to 9 in which the cofactor comprises catechol.
11. A composition or method according to any preceding claim in which the non-enzymatic bifunctional crosslinking agent comprises glutaraldehyde.
- 15 12. A composition or method according to any preceding claim in which the non-enzymatic bifunctional crosslinking agent comprises a di-isocyanate.
13. A composition or method according to claim 12 in which the di-isocyanate is Trixene.
- 20 14. A composition or method according to any preceding claim in which the non-enzymatic bifunctional crosslinking agent comprises a quinone.
15. A composition or method according to claim 14 in which the quinone is a benzoquinone.
- 25 16. A composition or method according to any preceding claim in which the phenol oxidase and the phenol hydroxylase is a tyrosinase.
17. A composition or method according to claim 16 in which the tyrosinase is a mushroom tyrosinase.

18. A composition or method according to claim 17 in which the mushroom tyrosinase is *Agaricus bisporus* tyrosinase.

19. Use of a composition or method according to any preceding claim for binding substrates together.

5 20. Use according to claim 19 in which the substrates are non water-absorbent.

21. Use according to claim 19 in which the substrates are water absorbent.

10 22. Use according to claim 19 in which the substrates comprise a non water-absorbent substrate and a water absorbent substrate.

23. Use according to claim 20 or 22 in which the non water-absorbent substrate or substrates comprise at least one of metal or plastic.

15 24. Use according to claim 21 or 22 in which the water absorbent substrate or substrates comprise at least one of wood, leather, cotton, paper, carpet, or textile.

25. Use according to claim 19 as a binder of particulates.

20 26. Use according to claim 25 in which the particulates comprise at least one of sand or glass fibre.

27. Use according to claim 19 as an undercoat to a coating.

28. Use according to claim 27 in which the coating is a paint.

25 29. Use according to claim 27 in which the coating is an adhesive.

30. Use according to claim 19 as a suture for closing a wound.

31. Use according to claim 30 in a method for closing a wound.

32. Use according to claim 19 as a gelling agent in food products.

5 33. A pharmaceutical composition comprising a pharmaceutically active ingredient and a crosslinked adhesive composition according to any of claims 1 to 3 or 7 to 18.

10 34. A kit for manufacture of an adhesive, the kit comprising separate components, wherein admixture of the separate components forms an adhesive composition according to any of claims 1 to 3 or 7 to 18.

15 35. A kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising: either

a non-enzymatic bifunctional crosslinking agent; or
a phenol oxidase and a phenol hydroxylase and optionally a cofactor;

20 wherein admixture of the first and second components forms a composition according to any of claims 1 to 3 or 7 to 18.

25 36. A kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising a non-enzymatic bifunctional crosslinking agent and a phenol oxidase and a phenol hydroxylase and optionally a cofactor, wherein admixture of the first and second components forms a composition according to any of claims 1 to 3 or 7 to 18.

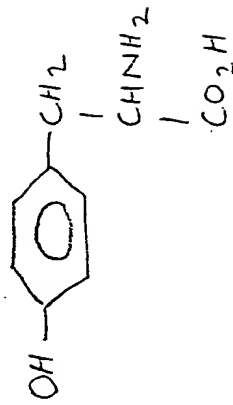
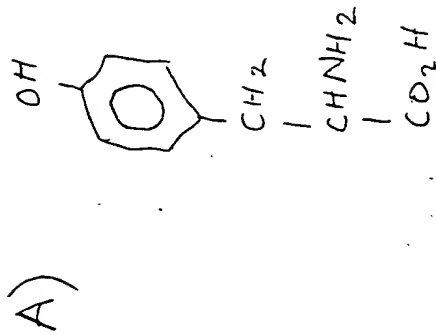
30 37. A composition for use as an adhesive substantially as described with reference to figure 2 of the accompanying drawings.

38. A method for forming an adhesive substantially as described with reference to figure 2 of the accompanying drawings.

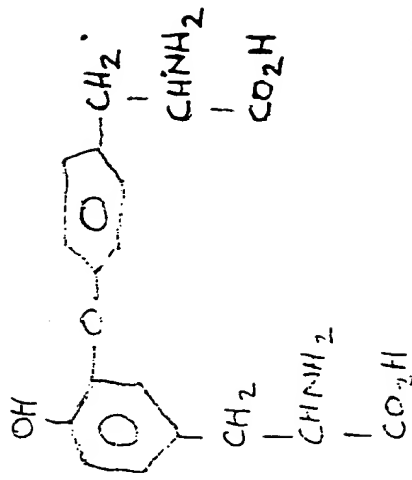
5 39. A kit for manufacture of an adhesive substantially as described with reference to figure 2 of the accompanying drawings.

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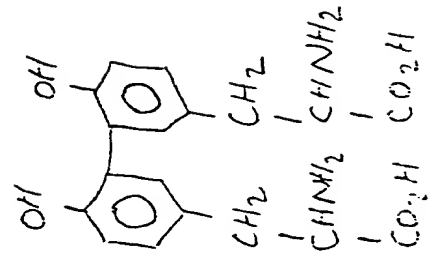
Figure 1



Hydrogen peroxide and
Peroxidase



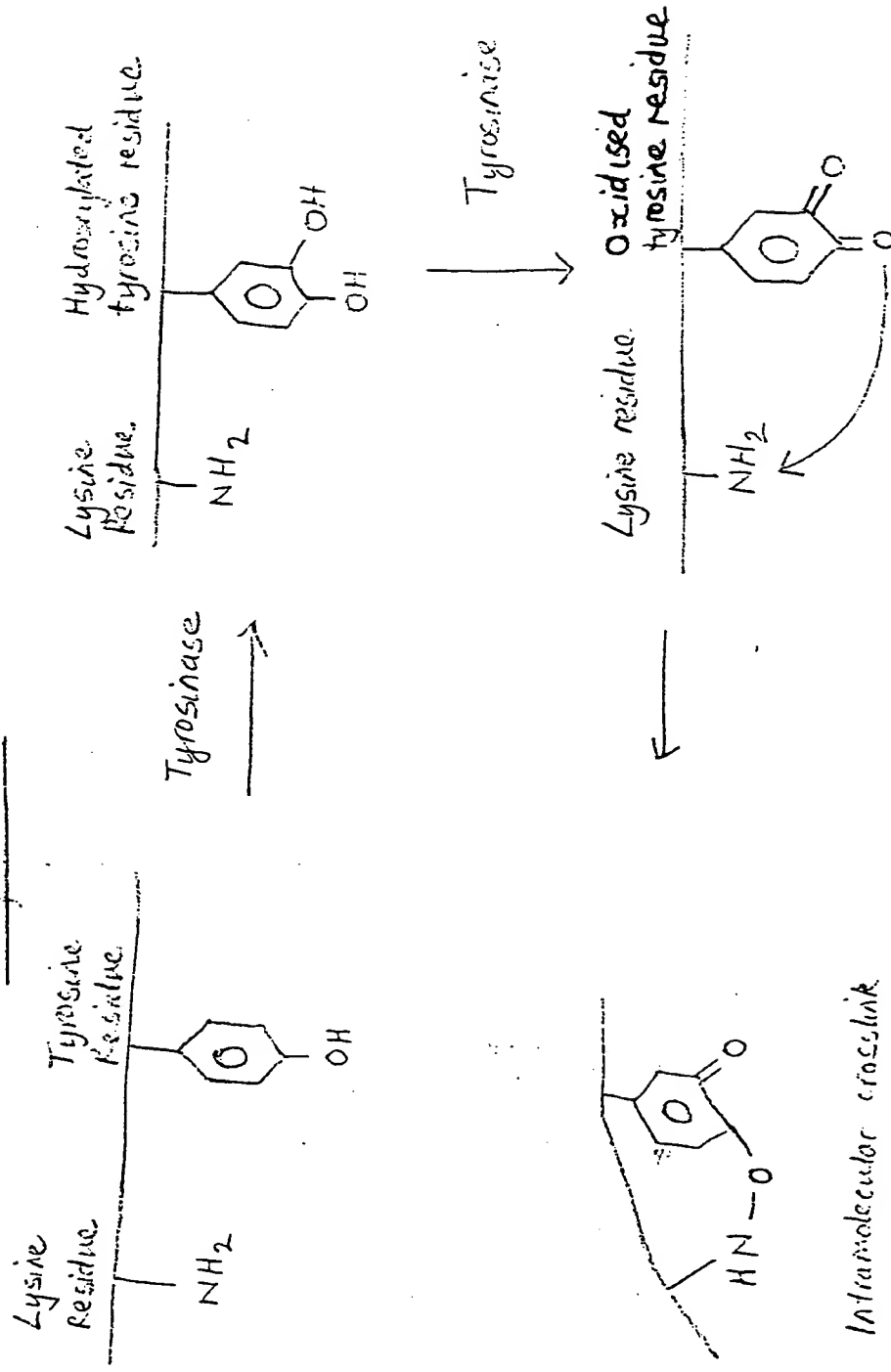
Isodityrosine crosslink



Dityrosine
crosslink

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Figure 2



Intramolecular crosslink
formed between oxidised tyrosine
residue and ϵ -amino group of
lysine residue

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Reddie & Rose

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